

## CLAIMS

- 1 1. An isolated recombinant human arginase I having substantially the same  
2 amino acid sequence as set forth in Fig. 2C and having a purity of 80-  
3 100%.
- 1 2. The recombinant human arginase I according to claim 1 further having six  
2 additional histidines attached to the amino terminal end thereof.
- 1 3. The recombinant human arginase I according to claim 1 or 2 having a  
2 specific activity of at least 250 I.U./mg.
- 1 4. The recombinant human arginase I according to claim 3 having a specific  
2 activity of 500 to 600 I.U./mg.
- 1 5. The recombinant human arginase I according to claim 4 comprising  
2 modification resulting in an *in vitro* plasma half-life of at least  
3 approximately 3 days.
- 1 6. An isolated recombinant human arginase I according to claim 1 or 2 having  
2 a purity of at least 90%.
- 1 7. The recombinant human arginase I according to claim 5, wherein said  
2 modification is pegylation.
- 1 8. The recombinant human arginase I according to claim 7, wherein said  
2 pegylation results from covalently attaching at least one polyethylene  
3 glycol (PEG) moiety to said arginase using a coupling agent.
- 1 9. The recombinant human arginase I according to claim 8, wherein said  
2 coupling agent is selected from the group consisting of 2,4,6-trichloro-s-  
3 triazine (cyanuric chloride, CC) and succinimide propionic acid (SPA).
- 1 10. A method of producing recombinant protein comprising:  
2 (a) cloning a gene encoding said protein;  
3 (b) constructing a recombinant *Bacillus subtilis* strain for expression of  
4 said protein;  
5 (c) fermenting said recombinant *Bacillus subtilis* cells using fed-batch  
6 fermentation;  
7 (d) heat-shocking said recombinant *Bacillus subtilis* cells to stimulate  
8 expression of said recombinant protein; and  
9 (e) purifying said recombinant protein from the product of said  
10 fermentation.

- 1 11. The method according to claim 10 wherein said *Bacillus subtilis* is a  
2 prophage.
- 1 12. The method according to claim 10 or 11 wherein said protein is human  
2 arginase I.
- 1 13. The method according to claim 12 wherein said human arginase I has six  
2 histidines linked to the amino-terminus thereof, and said purifying step  
3 comprises affinity chromatography in a chelating column.
- 1 14. The method according to claim 12 wherein said fermenting step is  
2 performed using a feeding medium consisting essentially of 180-320 g/L  
3 glucose, 2-4 g/L  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 45-80 g/L tryptone, 7-12 g/L  $\text{K}_2\text{HPO}_4$  and  
4 3-6 g/L  $\text{KH}_2\text{PO}_4$ .
- 1 15. A pharmaceutical composition comprising an isolated and substantially  
2 purified arginase.
- 1 16. The pharmaceutical composition according to claim 15 wherein said  
2 recombinant human arginase is human arginase I.
- 1 17. The pharmaceutical composition according to claim 15 wherein said  
2 recombinant human arginase is human arginase I containing six additional  
3 histidines attached to the amino terminal end thereof.
- 1 18. The pharmaceutical composition according to claim 15, wherein said  
2 composition is further formulated in a pharmaceutically acceptable carrier.
- 1 19. The pharmaceutical composition according to claim 15, wherein said  
2 formulation of said pharmaceutical composition is in a form suitable for  
3 oral use, for a sterile injectable solution or a sterile injectable suspension.
- 1 20. The pharmaceutical composition according to claim 16, wherein said  
2 recombinant human arginase I has a specific enzyme activity of at least 250  
3 I.U./mg.
- 1 21. The pharmaceutical composition according to claim 20, wherein said  
2 recombinant human arginase I has a specific enzyme activity of 500 to 600  
3 I.U./mg.
- 1 21. The pharmaceutical composition according to claim 16, wherein said  
2 recombinant human arginase I has a half-life in said patient plasma of at  
3 least 3 days.
- 1 22. The pharmaceutical composition according to claim 21, wherein said  
2 recombinant human arginase I has a half-life in said patient plasma of  
3 approximately at least 1 day.

- 1 23. The use of the human arginase I of claim 1 for the preparation of a  
2 medicament.
- 1 24. The use according to claim 23 wherein said medicament is used for the  
2 treatment of human malignancies.
- 1 25. The use according to claim 24 wherein said human malignancies are liver  
2 tumour, breast cancer, colon or rectal cancer.
- 1 26. A method of treatment of human malignancies comprising administering  
2 recombinant human arginase into a patient.
- 1 27. A method of treatment of human malignancies in a patient comprising  
2 administering a pharmaceutical composition that reduces the physiological  
3 arginine level in said patient to below 10  $\mu$ M for at least 3 days.  
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